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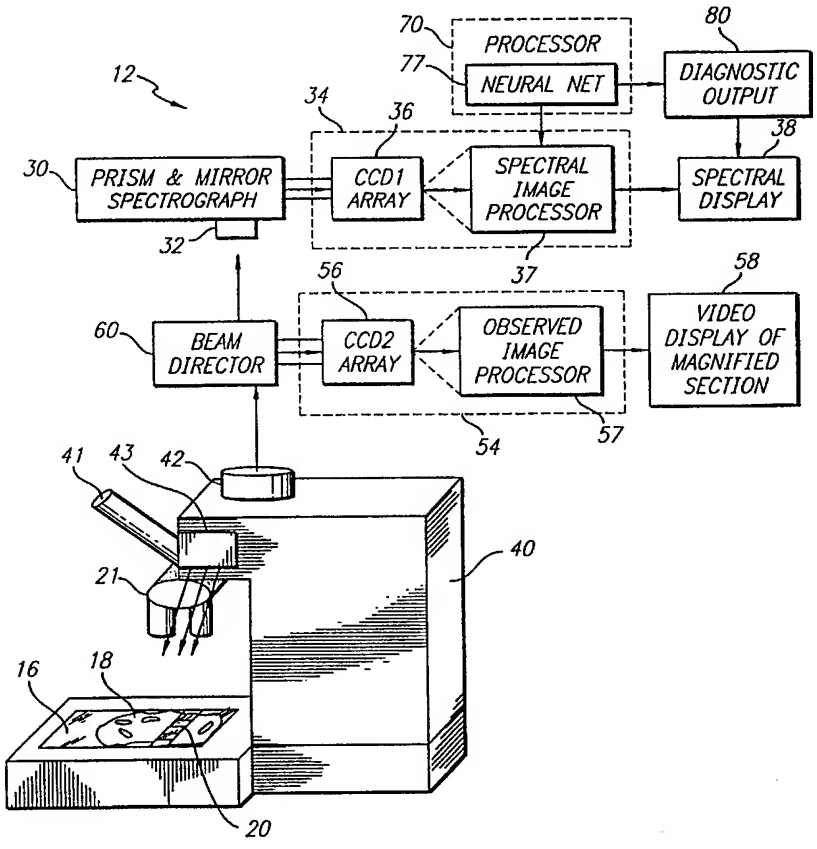
(54) **Title:** SPECTRAL TOPOGRAPHY OF MAMMALIAN MATTER

(57) **Abstract**

A multispectral topography system and method is provided whereby mammalian matter (18) is illuminated with a light source (43), and an image of a section (20) of the matter is transmitted to a multispectral imaging prism and mirror spectrograph (30) which substantially simultaneously, spectrally disperses the transmitted light. The spectral image is then acquired and prepared for further processing. The processor (37), typically resident in a computer system, processes the digital spectral data with a neural network to provide a diagnosis of the matter. The light source may be filtered light that is absorbed by specially prepared matter that reemits light (i.e. fluoresces). The matter could be pathological specimens to be magnified and analyzed by a microscope (transmission or fluorescent), or, alternatively, in vivo (live), whereby the image is transmitted by another conventional image transmitter, such as an endoscope. The system is also capable of displaying the transmitted visual image as well as the spectral image in conjunction with the diagnostic output.



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SPECTRAL TOPOGRAPHY OF MAMMALIAN MATTER

FIELD OF THE INVENTION

This invention relates to clinical pathology and more particularly to systems and methods that assist in the automatic analysis and diagnosis of diseased cells and tissue.

BACKGROUND OF THE INVENTION

Pathologists study samples of tissue and cells for the presence of malignancies and other diseases and abnormalities. As described below, recently, microscopy, spectroscopy and digital image processing, three traditionally distinct disciplines, have been coalescing to result in clinical pathology tools that more rapidly, automatically and accurately assist the pathologist to analyze and diagnose these conditions than was possible with conventional microscopy alone.

1. Microscopy

In one method of practicing conventional histopathology and cytopathology, after a biopsy of tissue or a smear of cells is removed from the suspect area of the patient and prepared on one or more slides, the pathologist studies the specimen under a light, or transmission, microscope. Tissue biopsies are often sliced into very thin sections and stained using one of, or a combination of, well-known staining techniques, such as H and E (hematoxylin & eosin) staining. Cell smears are similarly stained. Areas of abnormality are visually compared with representations of known disorders to determine whether and what disorders are presented.

The fluorescent microscope is another diagnostic tool often used by pathologists. Instead of passing incandescent, "white" light through the specimen, the illuminating "high energy excitation" light, from a source such as a Xenon or mercury lamp, is passed through one or more filter sets that passes only certain wavelengths of light onto (not through) the specimen. In response to the light incident upon it, the specimen, usually, but not necessarily, stained, then variously fluoresces, or produces lower-energy emissions of measurable wavelengths (colors) and intensities, thus providing diagnostically valuable information about the tissue or cells.

Fluorescent microscopy techniques can be classified into two categories, namely, those that involve endogenous (or natural) fluorophores, or reactions, and those that are exogenous (originating from outside the specimen). The former includes two types of reactions, namely, autofluorescence, whereby tissue compounds, such as elastin and collagen, naturally fluoresce without any augmentation from outside the specimen, and

immunofluorescence, whereby naturally fluorescing antibodies locate antigen in the tissues by combining with the specific antigen for that antibody (antigen-antibody reaction) and naturally fluoresce upon such combination.

The latter implies fluorescent tagging, or labeling, of a cell. In this technique, a molecule, such as a peptide, protein or antigen, that attaches to a highly specific target is "tagged" with a fluorophore such as fluorscein in order to detect whether there is a positive interaction. In one method, for example, a fluorescein conjugated monoclonal antibody is used to identify a specific antigen. This technique is often used to assist in the identification and diagnosis of nuclear-based diseases, such as viruses, but may also be used to identify bacterium and malignant tissue, for example. Another technique in this category, fluorescent in situ hybridization ("FISH"), takes advantage of either a) a paired-nucleotide interaction between a labeled probe (the "antisense strand") and endogenous mRNA (the "sense strand"), or b) a protein-protein interaction, whereby proteins are labeled and incubated with tissues that contain target binding proteins or receptors. Exogenous reactions also includes general fluorescent staining, such as the application of a auramine/rhodamine preparation to smears suspected for disease, such as tuberculosis. Such staining can highlight a specific feature such as a nuclear membrane from cytoplasm.

2. Image Processing

The increased processing power, speed and miniaturization of digital systems and computers have revolutionized the field of pathology. While the pathologist previously relied exclusively on the microscope and his or her own eyesight and experience to diagnose pathological disorders, current imaging and processing technologies have greatly enhanced the accuracy and speed with which today's pathologist may diagnose. For example, the charge-coupled device (CCD) array has been coupled to the microscope to enable the high resolution capturing of data representing microscopic images. These video images may be digitized by an analog-to-digital (A-D) converter, then displayed on a display, magnified or otherwise manipulated, and stored on other digital media. The video image data may also be further processed by a variety of image processing software. Neural networking, which is a non-linear, system that sorts out patterns from the data with which it is presented and learns from discerning and extracting the mathmenatical relationships that underlie the data, is one such processing structure. Applied to pathology, neural network systems compare specimen data collected by a CCD array to learned patterns of data representative of healthy and diseased tissue and cells in order to automatically characterize and assess the specimen for particular disorders.

3. Spectroscopy

Spectroscopy is the study of the spectral characteristics of objects, and more particularly, the study of the component parts (individual wavelengths) of the light of objects and the intensity of those wavelengths. It has long been used as a tool in the field of chemistry for identifying elements since each element possesses it own unique spectra. Since it was first realized that it can provide detailed information about both the chemical and physical nature of matter, spectroscopy has shown great promise for the field of medical diagnostics as well. For example, it has been demonstrated that certain spectral characteristics, such as fluorescence, indicate the presence of a malignancy or the metabolic condition of tissue. This information can be correlated to the object's location in the target field of view. It is often possible to determine the boundaries of indistinct edges by correlating spectra with "clustering" of spectral objects. Thus, this field has recently become a valuable partner with microscopy in order to analyze the spectral characteristics of a given suspect pathological sample.

Unfortunately, the potential for spectroscopy to enhance, and even revolutionize, the field of medical pathology has not yet been fully exploited. First, as discussed, the conventional fluorescent microscope uses one filter to block all but a single wavelength of light to excite the specimen, and another filter that permits only the reemited light (and blocks the higher energy excitation light) to pass to the optical output. Since the specimen is usually stained with a fluorophore that fluoresces at a single wavelength in the spectrum, this method provides useful diagnostic information about the specimen at this single wavelength only.

In order to gather more, and more meaningful, information about the specimen, the entire image must be captured again at a second wavelength through a second set of filters. This process is repeated many times until the desired number of spectral frames collectively obtain the "spectral envelope" of the object. Each frame is stored in a computer and the composite image can analyzed by the processing software described above and/or displayed on a display. This is known as multispectral imaging ("MSI").

However, this particular technique for MSI is impractical for numerous reasons. First, it requires the availability of numerous, costly filters. Second, exchanging filter sets in and out of a fluorescent microscope is time consuming. Third, the specimen must normally be stained with numerous dyes that cause the specimen to fluoresce at each excitation wavelength, thus risking the occurrence of specimen "bleaching," a phenomenon that can ruin or degrade the diagnostic value of the specimen.

Several new automated filter systems are available, such as rotatable filter wheels, acousto-optic tunable filters (AOTF), liquid crystal tunable filters (LCTF), and the

interferometer, all of which capture an entire image at each wavelength sequentially until the entire spectrum has been acquired. However, these systems are very costly, and the data processing of these images is an enormous task considering that a typical 512 by 512 pixel array, capturing, for example, 80 wavelengths, results in 21 MB of data per scene. Due mainly to complex and time consuming data processing, it is not uncommon for investigators to feel compelled to reduce the number of wavelengths acquired. Although 80 wavelengths may appear to be a large number, it is not uncommon for analytical chemists to acquire up to 1024 wavelengths simultaneously with off-the-shelf CCD detectors in a laboratory setting. Thus, acquiring and storing so many consecutive frames is impractical for analysis of specimens that are prepared with many fluorophores (which also risks photo-bleaching the specimens), or when computation speed is required or desired. In addition, the physical cost of these instruments is extremely high and shows little evidence of decreasing. Further, the above methods require the target object to be stationary and suffer no chemical change due to the environment during spectral acquisition.

Point spectroscopy is one known method of obtaining spectral data from an object. This technique captures the entire spectrum of, as its name suggests, only a single small point of an object at a time. In order to be practical and meaningful for the field of medical pathology, however, a spectroscopy system must be capable of spectrally dispersing, displaying and analyzing an entire specimen, or at least substantial sections of a specimen. Thus, point spectroscopy is not an ideal solution to the aforementioned drawbacks.

Thus, there is a definite need for a low cost system capable of rapidly and efficiently obtaining a meaningful quantity of MSI data of mammalian matter, including pathology specimens, and that is capable of automatically analyzing this data for the identification of disease in the matter.

SUMMARY OF THE INVENTION

The present invention addresses these need by providing a system and method that automatically assesses mammalian matter, notably one or more cells or tissue, for evidence of acute disease via multispectral acquisition of images of the matter. In the broadest embodiment, the system has three primary components, namely, an image transmitter, a unique multispectral imaging spectroscopy subsystem and a processor. The image transmitter includes a source of light that illuminates the matter and an optical output, and is adapted to transmit an image of a section of the matter to the optical output. The multispectral imaging spectroscopy subsystem is connected to the optical output and substantially, simultaneously spectrally

disperses the transmitted image into multiple component wavelengths to create a spectral image. The processor then processes the spectral image to provide diagnostic data representative of the image.

This system provides the advantages of obtaining, in a single acquisition, a complete multispectral image of, not just a single point, but a substantial section of the matter, while eliminating the need for sequential filtering.

In a more detailed embodiment, the multispectral imaging spectroscopy subsystem includes an imaging spectrograph and a charged coupled device (CCD) camera. The spectrograph has an entrance slit that permits the passage of light from a slice of the transmitted image of the section of the matter and a spectrum dispersing prism and mirror arrangement that disperses the light passed through the entrance slit into multiple component wavelengths of a predetermined spectral range to create a spectral image. The charge-coupled-device (CCD) camera is coupled to the spectrograph to acquire, and prepare the spectral image. The preparation of the acquired image typically entails two steps, namely, digitizing the image and pre-processing the digitized image with appropriate digital signal processing algorithms, as is well known in the art of image processing. The processor resides in a computer subsystem that processes the digitized spectral image and provides diagnostic data representative of the slice of the image. This system provides several important advantages. The spectrograph of this invention is a low cost device that eliminates the time intensive sequential capture of multiple wavelengths of a given portion of a specimen in favor of capturing the entire spectrum simultaneously. This enables rapid data processing which contributes to the system real-time diagnosis capability. The spectrograph, together with the digital camera and the computer data processing, provides an extremely simple, efficient and low cost tool for diagnosis of any suspect matter whose image can be transmitted to the spectrograph. Further, the entire system is a fraction of the cost of other MSI systems, such as the interferometer.

In an even a more detailed embodiment, the image transmitter is a lens-based image magnification system or telescope, broadly defined as any of various magnifying optical instruments. In one of these embodiments, the present also provides a low cost, efficient system that automatically assesses the pathology of prepared cytopathology and histopathology specimens via multispectral acquisition of images of those specimens whose images are illuminated, magnified and transmitted to the optical output via a microscope. The optical output of the microscope may also include a standard camera interface, such as a "C-mount" connection for rapidly connecting and disconnecting the imaging spectrograph thereto.

In yet a more detailed embodiment, the microscope includes an automatic x-y stage capable of controllably translating the slide for sequential MSI acquisition of the entire specimen. After one slice is acquired by the system, the computer-controlled x-y stage automatically sequences, or moves, the specimen so that the image of an adjacent slice of the specimen can pass the image-acquiring, entrance slit for spectral dispersion and acquisition of that slice. This process repeats until the entire sample, or as much as is desired, has been acquired and investigated.

In yet a further embodiment, a second CCD camera is provided in order to capture video images of the magnified sections of the specimen from the microscope and to provide the image for display and/or further processing. With this further embodiment, a beam directing assembly may be disposed between the microscope and the spectrograph in order to alternatively direct the magnified optical output to either the first CCD camera or the second CCD camera. In this way, the system is capable of providing the pathologist with two diagnostic tools in one, one being traditional video imaging and the other being spectral topography data, the latter provided in the form of graphical display, called spectral graphs, tabular form, an actual diagnosis printout, prognosis or suggestions (after such data is operated on by the neural network) and/or a combination of all of the above.

In an alternative embodiment, a beam splitter cube may be disposed between the microscope and the spectrograph in order to simultaneously direct the optical output from the microscope to both the spectrograph and the second CCD camera.

In a more preferred detailed aspect of the invention, when the microscope is set at 40x, the area of the sample submitted to the 5 mm long entrance slit is approximately 1.25 μm wide by 125 μm long. In this particular embodiment, the spectrograph is capable of acquiring spectral data at approximately each 0.5 μm along the slice. Each such 0.5 μm by 1.25 μm wide section is called an "object." With the specific design of the preferred spectrograph and CCD array capacity, a maximum of 240 objects can be captured simultaneously from each slice of the target sample. Further, each spectrum for each object contains up to a maximum of 740 wavelength data points in the 380 nm to 800 nm range, with a spectral resolution of 1nm at the 400nm wavelength to approximately 15 nm at 700 nm.

The neural network algorithm may alternatively comprise an unsupervised neural network (USNN), a supervised neural network (SNN), or a combination of the two. The USNN operates to automatically recognize and map (i.e. to train for) the presence of "fingerprint" spectra. The SNN may be implemented to act to automate the system and perform routine autocalibration.

One preferred method of spectrally analyzing matter for the presence of disease based upon a spectral analysis of the morphologic and physiologic deviation of the matter from the norm includes illuminating the matter with a light source, transmitting an image of the matter to a multispectral imaging spectrograph, spectrally dispersing the transmitted image through a prism and mirror arrangement into multiple component wavelengths of a predetermined spectral range, acquiring the spectrally dispersed image of the multiple component wavelengths, digitizing the acquired image and manipulating the digitized image, and processing the manipulated spectrally dispersed image to provide a diagnosis.

In an even more specific embodiment, the image transmitter provides a source of filtered light to the matter and transmits an image of fluorescent light reemitted from a section of the matter to the optical outlet. Typically, the matter comprises a prepared pathology sample and the image transmitter is a fluorescent microscope. In this specific embodiment, the present invention eliminates the need for multiple filters by acquiring the entire spectra of all fluorophores from a section of the fluorescing matter simultaneously.

A method of practicing this more specific embodiment entails illuminating the matter with filtered light of a specified wavelength to cause the matter to fluoresce, transmitting an image of a section of the fluorescing matter to an optical output, spectrally dispersing the transmitted image of the slice into multiple component wavelengths of a predetermined spectral range, acquiring and preparing the spectrally dispersed image and processing data representative of the image with a processor to classify the slice of the image into one of a preset number of categories indicative of the condition of the slice of the image.

The system and method of the present invention is effective in evaluating diseases through the analysis of the fluorescent features of mammalian cells and tissue that fluoresce either via endogenous or exogenous reaction. The former, also referred to as natural fluorescence, includes biological matter having an autofluorescing component, such as the elastin compound found in the aorta wall, arteries and other tissue. The analysis of such matter using the present invention yields diagnostically valuable information without any fluorescent staining preparations. The immunofluorescent method also falls into the endogenous category. In such case, the matter is typically prepared with at least one immunoglobulin antibody labeled with a tag, such as fluorescein, and is useful for the analysis of the kidney and heart, for example.

As an alternative to endogenously reacting matter, the matter, or specimen, may be treated with at least one fluorochrome. More particularly, the specimen may be treated with a histochemical probe labeled with a fluorochrome. Even more particularly, a fluorescent in situ

hybridization ("FISH assay") is performed on the specimen and the processor provides data indicative of the presence of at least one strain of one of a genetic disorder, malignancy, bacteria and virus, such as the Human Papilloma Virus (HPV). Alternatively, the specimen may be prepared with an immunohistochemical stain having immunohistological markers, including one of monoclonal and polyclonal antibodies conjugated to a fluorescent tag. One example of this is a specimen stained with a direct immunofluorescent assay (DIF) to identify the presence of chlamydia trachomatis (CT). Even more beneficially, the system is capable of simultaneously quantifying a specimen treated with multiple probes labeled with different fluorochromes, immunohistochemical markers or a combination of all of the above, in order to diagnose the condition that each stain or fluorophore probe, individually, is designed to isolate.

Thus, the present invention can provide a significant advance in the field of cervical cytology by providing a tool that automatically assesses a Pap smear or cervical biopsy for both various HPV strains and CT bacteria. In particular, a Pap smear specimen may be prepared using a both a FISH assay for HPV identification and with a direct immunofluorescent (DIF) stain directed to detect CT. In this way a single, dually stained PAP smear analyzed by the system of the present invention can provide rapid, robust and low cost identification of some of the primary cytological disorders and precursors to cervical cancer.

Conventional staining of biological matter to induce a fluorescent reaction is another category of reactions applicable to the present invention. For example, a smear may be tagged with well-known fluorescent stains, such as rhodamine/auramine to produce a fluorescent reaction that can be exploited by the present invention for the identification of several diseases, such as tuberculosis.

Other features and advantages of the present invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is basic schematic showing the automated multispectral topography system of the present invention wherein light transilluminates through a mammalian matter, such as tissue or cells;

FIG. 1B is a variant of the image transmitter of FIG. 1A, wherein the light incident on the matter is either reflected off of the matter or is absorbed by the matter which reemits light of a lower energy (i.e. fluoresces);

FIG. 2 is a schematic of a more detailed embodiment of the present invention wherein the matter is prepared on a slide and the image transmitter is a fluorescent microscope;

FIG. 2A depicts the matter prepared on the slide shown in FIG. 2 in greater detail;

FIG. 3 is a flow chart describing one preferred method of the present invention;

FIG. 4 is an observed video image of a section of a fluorescing, TB-positive, sputum sample stained with auramine/rhodamine, with a slice of the section bounded by a rectangle;

FIG. 5 is a false color representation of the spectra present in the slice shown in FIG. 4;

FIG. 6 is a more refined version of the spectral image of FIG. 5, after profiling with an unsupervised neural network;

FIG. 7 is a graph of 25 spectra representing the spectral fingerprint of the 25 channels of the section of the image shown in FIGS. 5 and 6;

FIG. 8 is a chart showing the unsupervised neural network presentation of the analysis of, in the two left columns, the calibration smear shown in FIGS. 4-7, and, in the right two columns, a patient sample;

FIG. 9 is a graph showing spectral curves of autofluorescing specimens, one being a portion of a healthy aorta wall and the other a degenerated aorta wall;

FIG. 10 is a graph showing the spectral curves of diseased native and transplanted kidneys;

FIG. 11 is a graph showing the spectral curves of three slices of an immunofluorescing kidney sample, each stained for IgA, IgG and IgM, respectively; and

FIG. 12 is a graph showing the spectral curves of sections of two heart biopsies whereby no rejection is recorded for either section.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention summarized above and defined by the enumerated claims may be better understood by referring to the following detailed description, which should be read in conjunction with the accompanying drawings. This detailed description of particular preferred embodiments, set out below to enable one to build, use and practice particular implementations of the invention, is not intended to limit the enumerated claims, but to serve as particular examples thereof. The particular examples set out below are preferred specific implementations of a system that provides multispectral topography for mammalian matter, i.e,. cells and tissue, namely, one that provides automated spectral data acquisition, analysis and diagnosis of images

of matter that is suspected to be diseased. The invention, however, may also be applied to other types of mammalian matter, such as that which is not suspected to be diseased, but identification of its morphological and physiological characteristics is nonetheless desirable. Further, in the presently described invention, the matter is illuminated with a source of light, with one specific source providing filtered light to enable analysis of the fluorescent characteristics of the matter.

Before describing the invention in greater detail, namely, the method of multispectral analysis employed by the present invention, its various components and experimental results, we now explain the theory behind the application of spectral topography as a tool for revealing the "hidden morphologies" and physiologies of tissue and cells and its implications for medical pathology.

I. Morphologic Effects On Normal And Abnormal Cells and Tissue

It has long been recognized that the study of the form, such as size and shape, of cells and their environments (cytomorphology) and tissue biopsies can reveal diagnostically valuable information regarding the state of those structures. General Biologic activity is reflected best in the cellular structures of the nucleus. Functional activity is reflected mainly in the morphology of the cytoplasm. The "healthy," baseline morphology of cells may be considered a reference level, called euplasia, which is the form and structure of normal cells absent stresses from pathologic processes. In euplasia, key nuclear structures may appear under a light microscope as round or rounded, uniform and having regular patterns of nuclear components such as chromatin, and possess a degree of predictability from one nucleus to another. In tissue analysis, predictability from one cell to another cell of the same type would be expected.

Numerous morphological effects of processes associated with carcinomas and precursors to the same have been identified. For example, significant irregularities in the shape of nuclear membranes of cells, disarray in the structural orderliness and shape of chromatin and parachromatin (the pale areas of the nucleus), the enlargement and irregularity of the shape of nucleolus, and importantly, an increased ratio of nuclear area to cytoplasmic area (N/C ratio) are some of the recognized morphological factors which favor a finding of cancer.

Unfortunately, to date, such morphological analysis under a transmission or fluorescent microscope has, by itself, been of limited value in diagnosing diseased cells and tissues for several reasons. First, morphological changes in cells and tissue occur in varying degrees. Many changes cannot be recognized (either clearly or at all) through the eyepiece of a light microscope even by an experienced pathologist, or even with the manipulation of video

images of slides of specimens. These hidden morphologies are due to either the inherent nature of the changes, the stage of morphologic activity or a combination of the two. Second, to date, there has been no observable absolute morphologic feature of cancer - or a malignant criteria - that when present, unequivocally reveals that the particular cell or tissue under observation is cancerous or, when absent, means that there is no cancer.

The present invention is employed to reveal the hidden morphologies of suspect cell and tissues, whether in vivo or prepared specimens, by illuminating them with a light source, and analyzing their spectral content with the aid of a unique multispectral imaging spectrograph and sophisticated processing algorithms.

II. The Method and Components

1. Push Broom Multispectral Imaging

There are several traditional ways of acquiring multispectral information from a remote field of view. As discussed above, one method acquires an entire field through a series of wavelength filters. The number of filters will be equivalent to the number of wavelength data points needed to identify the spectral signatures of each component in the field. The field of view is fixed so data cannot be acquired if the object moves or changes in any way.

In another method developed for remote earth monitoring and implemented by the present invention, the system acquires a small slice of the field and passes it through a specialized wavelength dispersive spectrograph that acquires the entire spectrum of the slice simultaneously. To cover the entire field it is necessary to move on to the next, adjacent slice. Concatenating each acquisition enables the entire field to be covered. This method is often referred to as "push broom" spectral topography because the sample is "pushed" across the spectrograph entrance aperture, or slit, and is, in numerous ways, more versatile and efficient than the prior described method of multispectral imaging acquisition.

The present invention uses push broom multispectral image acquisition of suspect mammalian tissue and cells to reveal, in the case of specimens prepared on slides, their hidden morphologies and, in the case of in vivo analysis, their hidden physiologies, and to ultimately provide clinical diagnosis of the tissue and cells.

2. The Basic Components and Method

FIG. 1A is a block diagram showing the basic components of the system. An image transmitter 1 includes a light source 2 and an optical output 3. The light source 2 illuminates the mammalian matter 4 to be analyzed, whose image is transmitted to the optical output 3. An imaging spectroscopy subsystem 6 substantially simultaneously spectrally disperses the transmitted image into multiple component wavelengths of a given range. This method of

spectral dispersion uses a spectrograph originally designed for remote, telescopic earth monitoring and astronomy and is currently in use for both applications in military and civilian environments. The original spectrograph was patented to Warren et al. (patent no. 5,127,728) and was designed for use in the infrared wavelength range of 3 to 15 μm, and is incorporated herein by reference. For the life sciences applications of the present invention, the optics were redesigned for use in the primarily visible 360 to 800 nm wavelength range. A processor 8, such as a PC computer loaded with the appropriate software, operates on data representative of the spectrally dispersed image to ultimately provide a diagnostic output 8 relating to the condition of the matter 4. This diagnostic output 10 could be provided at a computer screen, at a printout, or at any conventional output device.

FIG. 1A depicts an image transmitter arrangement whereby the light source 2 transilluminates through the matter 4. This is typically the case when, for example, a transmission microscope is the image transmitter that provides white light for a relatively thin specimen, such as a cell smear, or a very thin tissue biopsy. FIG. 1B shows an alternative arrangement for the image transmitter 1, which is representative of one of two optical scenarios. In one scenario, the light from the source 2 is reflected off of the matter 4 and to the optical output 3. Reflection could be used in several situations, one being where the suspect tissue biopsy is too thick for the light to meaningfully transilluminate therethrough, and another being where the matter to be analyzed is not removed from the patient, but is rather in vivo. In the second scenario represented by FIG. 1B, the light from the source is filtered, and this filtered light 2 is absorbed by the matter 4. In response, the tissue or cells, reemit light of a lower energy level. This fluorescent light is provided to the multispectral imaging spectroscopy subsystem for dispersion and analysis.

FIG. 2 is a schematic of a more detailed embodiment of the present invention wherein the image transmitter is a fluorescent microscope. The primary components of this multispectral topography system 12 include a fluorescent microscope 40, a prism and mirror imaging spectrograph 30, a first CCD camera 34, a processor 70 and a diagnostic output device 80.

The fluorescent microscope 40, found in many laboratories and research facilities, has an eyepiece 41 and an optical output 42 having a standard camera interface, such as a video port with a "C-type" mount. The light source 43 of the microscope is filtered by internal filters and is absorbed by a specimen 18 which has been prepared on a slide 16. The specimen then reemits light of a lower energy level and of varying intensities across the specimen, and lenses magnify the image of a section of the fluorescing specimen 18 and supply such image to the

both the eyepiece 41 and optical output 42. The specimen is typically prepared using conventional dyes and/or labeled with conventional tags, using conventional techniques, but need not be. A spectroscopy subsystem connected to the microscope 40 includes the prism and mirror, wavelength dispersive imaging spectrograph 30 having an entrance slit 32. The slit permits the image of a slice 20, as seen in greater detail in FIG. 2A, of the specimen 18, the slice itself comprising many "objects" 21, to pass therethrough and into the spectrograph 30, and a first CCD camera 34. The novel and modified spectrograph 30 provides good image quality over a broad range of operating wavelengths simultaneously, allowing large spectral intervals to be surveyed without moving any of the elements of the system.

A beam directing assembly 60, also called a "beam splitter," constructed with a "flip" mirror 62, provides both a video image and spectral acquisition. Thus, when the mirror is flipped in one direction, the spectrally dispersed light is focused on, and acquired by, the first CCD matrix array 36 of the camera 34. As done in conventional CCD cameras, the analog image is then prepared for viewing and further processing. In particular, the image is digitized by an analog-to-digital (A to D) converter and is then manipulated, or pre-processed, by the spectral image pre-processor 37, often called a digital signal processor (DSP). The prepared spectral image can then be fed into the processor 70, as discussed in detail below, and/or displayed on a display 38 such as a CRT, LCD screen, or other conventional display.

When the flip mirror 62 is rotated to a second orientation, the visual, microscopically magnified image, i.e. a video image, is captured by the matrix array 56 of the second CCD camera 54. This image is also digitized by an A-to-D converter and advanced image processing algorithms 57 (another DSP) manipulate the image for high quality display on a conventional display 58. Conventional image pre-processing algorithms in DSP's include smoothing, normalization, background substraction, principal component analysis (PCA) and partial least squares (PLS). In this way, both spectral imaging data and visual imaging data of the specimen can be displayed for the pathologist, stored, analyzed and/or further manipulated. In an alternative embodiment not shown, a beam splitter cube replaces the beam directing assembly 60 to send light simultaneously to the spectrograph 30 and the observed-image CCD camera 54.

The spectrograph 30 uses a prism made of inexpensive flint glass. The support and body of the unit may be in either cast aluminum, or ribbed metal plate, or any other material that provides for lightweight and for enhanced rigidity. The optical system is fully ray-traced. The system, with or without the beam director 62 is easily assembled to the optical output of

a conventional microscope, typically a video port, with the use of a standard "C-type" mounting or any other acceptable connecting means.

In one preferred embodiment, when the fluorescent microscope is set at 40x magnification, each slice 20 captured through the slit 32 and by the spectrograph 30 is approximately 1.25 μm wide by 125 μm long. Further, the system is capable of acquiring spectral data at approximately each 0.5 μm along the slice 20. Each 0.5 μm by 1.25 μm wide section is called an "object" 21. With the specific design of the preferred spectrograph and CCD array capacity, a maximum of 240 objects can be captured simultaneously from each slice of the target sample. Further, each spectrum for each object contains up to a maximum of 740 wavelength data points in the 380 nm to 800 nm range, with a spectral resolution of 1nm at the 400nm wavelength to approximately 15 nm at 700 nm. It is understood, however, that other slit sizes and CCD cameras having other resolution capacities may be used, resulting in different (and greater) object and spectral envelope resolutions.

Sequentially scanning the entire image of the specimen 18 is achieved by scanning the microscope stage under computer control in the histology or cytopathology setting. The first CCD matrix array detector 36 collects individual spectra along rows of pixels from objects located in the entrance slit 32. This format is sometimes referred to as an "open image" because there are no restrictions on the area of the object to be examined and is certainly the least expensive and most flexible method for pathology samples subject to microscopic examination.

All spectra of all objects in a slice are acquired simultaneously in milliseconds, depending on signal strength. Photo-bleaching is reduced especially if objects such as single cells or glomeruli are selected by automated pattern recognition and correlated with signal strength at certain wavelengths.

The processor 70, which will typically be part of a computer system, such as a PC, contains a powerful neural network 72 that provides near instantaneous recognition of the spectral fingerprints of the objects 21 of the specimen 18. In one preferred embodiment, there are up to 240 objects, each as small as 0.5 μm, that the neural network can substantially simultaneously process. The relatively small file size for each acquisition greatly enhances computation speed and simplifies memory management.

FIG. 3 illustrates how the push-broom methodology is applied to the present invention for multispectral analysis mammalian matter. In step 100, an image of the matter, whether prepared on a slide or in vivo, transmitted to the optical output. It is understood that, in the broadest embodiment, the image can be transmitted via any known means, such as transillumination through or reflection off the matter, or fluorescing from the matters. In the

case of microscopy (transmission or fluorescent), a particular field, or section, of a magnified specimen is presented to the entrance slit 32 of the spectrograph 30. In step 102, a single slice 20 of the section, containing up to 100 objects 21, passes through the slit 32 and, in step 104, strikes the first curved surface of the prism, is refracted, strikes the second surface, and exits to strike the spherical mirror as is described in the Warren et al. patent. The wavelength dispersed light then returns through the prism to be focused and stored onto a first CCD matrix array, step 106, which acquires and prepares the light. As used herein, the preparation may entail digitizing and pre-processing the spectrally dispersed light, but not necessarily. The pre-processing, or manipulation, of the digital image is accomplished with conventional DSP algorithms to improve its appearance. Over 80% to 90% of all light, over the entire wavelength range, is transmitted through the system.

The processor then processes the spectral data representing the spectral envelopes of each object in the slice, step 108, using the neural network that sorts out the morphological patterns in the objects of the cell(s). The system then inquires into the status of the acquisitions, step 110. If only a single acquisition is needed or desired, the process halts in step 112 and the diagnostic data may be output for review by the pathologist. However, if as is usually the case, an additional slice of the specimen is to be spectrally dispersed and analyzed, i.e. the answer to inquiry 110 is "no," and an adjacent slice of the image is transmitted through entrance slit, step 114. In the case of microscopy, the specimen slide is moved by the x-y stage of the microscope 40 to permit the passage of light representing a slice adjacent to the previously dispersed slice. Then, the process reverts to step 104 for spectral dispersal, acquisition and analysis of the objects in that second slice. This process is repeated until the entire or enough of the specimen is analyzed in this fashion. Once this sequential acquisition process is complete and the spectral data for each object is stored in the appropriate memory "bins," as described below, the processor may then synthesize this data to produce a complete diagnosis for the specimen.

The advantages of utilizing push broom spectral data acquisition for cellular analysis are numerous. First, the diagnosis is practically instantaneous. To acquire enough spectral data using alternative techniques would require the creation of huge digital files prior to data processing. A full acquisition according one preferred embodiment of the present invention includes 240 spectra, each with 740 data points, yet is only 185KB. A file of this size is easily handled, resulting in very rapid data processing and decision making. Further, the entire system costs much less than other methods of analysis, such as the interferometer.

This method also enables fast low-resolution assessments to determine gross parameters prior to high-resolution scans. In addition to acquiring multispectral topographical maps, the system can "paint" the video image of the specimen with false color "fingerprint" spectra indicative of normal and diseased tissue. The system also differentiates between chronic and acute disease as well as evidence of anti-rejection drug toxicity.

3. The Neural Network

Traditional mathematical algorithms perform calculations sequentially, delivering results based on linear transformations. The neural network (NN) 72 performs calculations in parallel, in an analogous way to the human brain, to perform non-linear transformations. To date, the system has been powered by an unsupervised neural network (USNN). However, the system may alteratively be powered by two neural networks: a USNN and a supervised neural network (SNN). The USNN collects each spectrum from each object, characterizes it, sorts it, and places it into a "bin" of unique spectral signatures. It can thus be employed to automatically recognize and map (i.e. to train for) the presence of "fingerprint" spectra, i.e., to identify bins containing identical spectral signatures. The SNN may then be used to determine the special features that differentiate the spectra in each bin from those in other bins. Thus, the system can then compare the presenting spectra of the objects of the matter under analysis with the map to identify the presence of the disorder.

The USNN can be thought of as a "filter" or "sieve" that characterizes and sorts each spectrum as it is collected and places it into its morphological bin or "class" of similar spectral signatures. The process is analogous to alphabetizing the words in a book by combining those that are the same or have a common root. By the end of this procedure, the number of bins represents the number of spectral objects defined by their spectral-morphological characteristics. The process is referred to as "digital chromatography" because the function is almost identical to the process used in analytical chemistry for the separation of mixtures of chemical compounds. The big difference is that the USNN program adds the extra dimension of providing spatial information by mapping a particular class of spectrum back to the sample itself.

The operator can decide to combine some spectral classes, such as background features, or eliminate others. This is achieved by "thresholding" either by the user or the computer. This is a process that enables the USNN to delineate, identify and separate identical and non-identical spectra within user defined limits of the application. The incorporation of human "wet neurons" to set up the thresholds enables human experience to control the operating limits of the software. The USNN becomes a "white box" rather than a "black box"

because the sensitivity and the operating parameters of the network are available to operator influence at all times.

Once the USNN has self-trained, with initial well-qualified samples, the USNN compares each newly acquired spectrum against the spectral classes and categories it recognizes. The USNN identifies matches, near matches, and no match spectra in the new acquisition(s). Each class of spectrum is coded into a few bytes of data and stored in memory. Every future spectral acquisition is similarly coded and compared to the stored data. This process reduces a spectrum of 740 data points to a block of memory only a few bytes in size, consequently recognition of hundreds of spectra is performed in near real-time. Training can take up to two minutes for complex materials and a few seconds for simple spectra. After training, recognition is within a second.

The processor 70 may also incorporate a Supervised Neural Net (SNN) that works in conjunction with the USNN. The SNN would identify the special features of each bin, automates the system and performs routine autocalibration. Past experience has shown that combining these two neural net architectures is very effective for controlling many variables, some or all of which can change with time. Most humans tend to overlook or "accommodate" certain changes. In a multi-parametric system, such as cellular pathology, this is very dangerous because a change in one variable can result in non-linear affects elsewhere in the process and compromise the accuracy of an assessment. The two neural network systems could form a transparent alliance to automatically ensure that conditions necessary for accurate repeatable diagnoses are not compromised by changes in temperature, mechanical maladjustment or operator error.

One or, more likely, many systems of the invention may be trained and retrained by USNN's in order to spectrally characterize the morphologies of the cells of all presenting pathological disorders and diseases, thus creating a "library" of spectral fingerprints representative of those presenting conditions. It is expected that, eventually, the entire and finite universe of categories of pathological disorders will be spectrally characterized by USNN's. When this milestone is achieved, systems could then be equipped with SNN's which include databases containing the all pre-trained, spectral fingerprints of the various possible conditions. When a specimen whose pathology is unknown is presented to the system of the present invention, the SNN will rapidly compare and map its spectral fingerprint with that of all fingerprints stored in the database or, in the appropriate category of the database. With the availability of powerful, fast and low cost personal computer systems with large amounts of memory capacity, a single personal computer system will be capable of storing the an entire

"spectral library" thereby providing automatic diagnosis of the condition of any cellular or tissue specimen presented to it.

It is understood that those skilled in the art may develop protocols to enable specific histological assessments to be selected from a computer menu to simplify and minimize continuous human interaction with system, perform autocalibration, continuously monitor the status of the entire system, and record user operations.

4. The Outputs

The system of the preferred embodiment is capable of providing several diagnostic outputs. Figures 4, 5, 6, 7, and 8, discussed in greater detail below, illustrate some of these outputs from analysis of mammalian matter, namely, a section of a sputum sample from a patient positive for tuberculosis, wherein the sample is stained with the chemical dye preparation, auramine/rhodamine. The slide, viewed under epi-fluorescence, enabled the system to provide spectral characterization and mapping of the fluorescing tuberculosis bacteria. The neural network monitors intensity and when multiple spectra are presented (while not necessary for this example wherein only a single fluorophore and thus a single emitted wavelength is present) deconvolution requirements and paints back to a computer monitor the location on the sample of an identified and processed spectrum.

In particular, FIG. 4 shows a grey scale video, or "observed," image of a fluorescing and magnified section of the specimen on the video display 58. It is understood that the video display can be any conventional display, such as a CRT or LCD screen, or equivalent. FIG. 5 shows the same image with a false color representation of the spectra present down the slice. FIG. 6 is a more refined version of the spectral image of the slice identified in FIG. 5, but partitioned into "channels" after profiling with the USNN. FIG. 7 is a graph of 25 spectra out of a possible 240. The y-axis represents the intensity, or magnitude, of the light emitted from the object of the slice of the specimen across its spectrum. The entire baseline-to-baseline fluorescence envelope between 540 and 700 nm (peaking around 580 nm) is captured and relative intensity differences as a function of location on the sample clearly portrayed. FIG. 8 shows how the USNN associated each spectrum of similar nature to an object. In particular, it is a chart representing the objects in a training set on the left half and on the right, objects of another sample.

All of this visual information may assist the pathologist in analyzing and diagnosing the matter. Ultimately, however, the system, via the neural network, is designed to provide an actual diagnosis of the condition presented by the matter under observation. This diagnosis can

be provided on any conventional output medium. such as on a display, in hard copy, stored to memory, transmitted to another computer system, or any combination of the above.

III. Applying Multispectral Topography to Fluorescing Matter

Many fluorescent techniques have been found very effective in the analysis of a variety of types of biological mammalian matter. Some techniques exploit the endogenous characteristics of the particular matter (i.e. natural fluorescence), including autofluorescence (such as in the aorta wall and arteries) and immunofluorescence (for example, for analysis of kidney and heart tissue). Other fluorescent techniques that are not endogenous include immunofluorescence using fluorescence-labeled, monoclonal or polyclonal antibodies (immunohistologic markers), in situ hybridization techniques, and standard dye methodologies. It is thus understood that mammalian matter, as used in this section, includes any of the aforementioned types of matter prepared by any of these fluorescent techniques, and, in the case of matter that autofluoresces, may have no fluorescent preparation at all. In all of these cases, however, the analysis of such matter is greatly enhanced by the incorporation of the automated spectral topography system of the present invention. The remaining discussion details some specific applications of the present invention and the experiments conducted which verify its efficacy for diagnosis of pathological disorders.

1. Applied to Naturally Fluorescing Matter (Endogens)

Matter that is recognized to contain a substantial autofluorescence compound, such the elastin present in the aorta wall and arteries, or collagen present in other matter, may be analyzed by the present invention with no fluorescent staining to evaluate autofluorescent patterns of diseased and normal aortas and arteries and to yield basic, diagnostic information. Further, analysis of the natural fluorescence of cell and tissue matter from both native and transplanted organs, such as the kidney, heart and other organs, using an immunofluorescent staining technique, including fluoresceinated antisera to immunoglobulins, in order to diagnose both native diseases and rejection, can also be greatly enhanced by the use of the multispectral imaging spectrometer system of the present invention.

a. Applied to Autofluorescent Fluorophores

FIG. 9 shows the spectral features of two samples of aorta walls, which possess autofluorescent elastin layers. The samples were examined under an epi-fluorescence microscope excited at 436 nm and exhibited strong blue-green fluorescence. The spectral features provided by the spectroscopy subsystem of the present invention and shown in the figure clearly differentiate between a normal aorta wall (curve 1) and an aorta wall showing degeneration (curve 2). Ultimately, once the neural network has fully the trained the system

by many such samples, resulting in the identification and classification of the spectral characteristics of the entire universe of normal and degenerated aorta walls, the system of the present invention will be capable of analyzing and diagnosing any aorta wall sample of unknown diagnosis.

b. Applied to Immunofluorescence

The diagnosis of disease in some native organs, such as end stage renal disease (ESRD), and of some heart diseases, and of rejection of transplanted organs relies, in part, on the effective evaluation of immunofluorescence findings in frozen sections of biopsy specimens. For example, the ESRD population is growing progressively older. Both dialysis and kidney transplantation are effective techniques for prolonging life in ESRD. The commonly employed diagnostic tests (i.e., renal transplant ultrasound and hippuran scintigram) are helpful in differentiating rejection from other causes of graft malfunction. Pulsed-Doppler is a good tool for studying vascular complications involving renal transplants and helps differentiate vascular rejection from other complications. The diagnostic value of quantitative Duplex Doppler Sonography (DS) in renal allograft evaluation is being viewed increasingly critically. Therefore, renal biopsy to establish specific diagnosis by histopathology evaluation of allograft dysfunction remains mandatory.

In fact, specific renal parenchymal disease, such as acute or chronic rejection, "*de novo*" or recurrent glomerular disease, immunosuppression nephrotoxicity, (mainly by cyclosporine A) that contribute to graft malfunction, can typically only be diagnosed by renal histopathologic study.

Recognizing the importance of effective and efficient histopathologic analysis for these conditions, the system of the present invention was thus used to evaluate fluorescence patterns in organ specimens stained for immunoglobulins, complement components, and plasma proteins and was correlated with light microscopic and standard immunofluorescence findings and clinical data. It was found that spectral analysis of the following histopathological specimens using the system of the present invention provided qualitative as well as quantitative information that is extremely valuable in the diagnosis of these specimens.

i. Applied to Kidney Biopsies

The system of the present invention was tested and was found to assist in the early diagnostic evaluation of biopsies from patients with renal disease, and with routine monitoring of patients who have undergone transplantation.

In particular, immunofluorescence studies of renal allografts with rejection usually reveal non-diagnostic patterns of immunoglobulin deposits with existing fluorescence

microscopes. The application of the spectral topography microscope of the present invention has been shown to aid in the detection of immunoglobulin deposition as a function of their spectral fingerprints and their spatial correlation within the tissue and adds a powerful digital advance to current methods of observation.

In patients with native kidneys, spectral images from the system can be compared to clinical findings and standard light and immunofluorescence studies to categorize the disease state and quantifies its severity. Currently, immunofluorescence is graded crudely (0 to +4). The system adds quantitative as well as qualitative new insights into the structural abnormalities underlying immune and non-immune system based renal diseases.

The experiment entailed analyzing archival slides of patients with suspect renal or heart transplants or native organs presenting with evidence of disease. The samples had been treated with immunofluorescent stains specific to IgA, IgG or IgM. They were also treated with Hematoxylin/Eosin (H&E) staining to observe and analyze the samples under transmission (white light) microscopy. The system operating parameters were as follows:

The microscope objective:	40X
Entrance slit to the spectrometer:	50 μm
Average acquisition time of spectra:	9 Sec
Average acquisition time of Observed image:	1 Sec
Number of objects per acquisition:	240
Size of each object:	0.5 μm by 1.25 μm
Spectral range per acquisition:	400 to 800 nm
Number of Renal disease patients:	7
Number of repeat tests per slide:	6

The excitation wavelength was 405 nm and spectra were acquired through a long pass filter that allowed all light of greater than 470 nm to pass, and blocked the excitation wavelength and all wavelengths shorter than 470 nm. An Olympus BH2 epi-fluorescence microscope was used.

All slides had been previously examined and were photo-bleached to some extent, and grossly in some cases. Nevertheless, as shown below, the system provided evidence that spectral topography is able to differentiate between native kidney with chronic disease and

transplanted kidney showing evidence of chronic rejection or cyclosporine toxicity. It was also possible to differentiate between immunofluorescent staining for IgA, IgG and IgM.

(1). Comparison of native kidney with chronic disease and transplanted kidney with chronic rejection

FIG. 10 shows that spectral topography differentiates between native kidney with chronic disease, (curve 1) and transplanted kidney showing signs of rejection with and without cyclosporin toxicity, (curves 2 and 3). All these biopsy samples were stained with fluoresceinated anti-sera to human IgA:

Curve 1: (14058) Diabetic, chronic diabetic nephropathy, and glomerulosclerosis.
Curve 2: (12815) Diabetic, 7-year-old transplant with chronic cyclosporine toxicity and signs of rejection.
Curve 3: (13487) 1 year transplant, chronic transplant rejection, glomerulopathy and segmental sclerosis.

(2). Differentiation between IgA, IgG and IgM

FIG. 11 shows clear differentiation between the spectra of immunofluorescence staining for IgA, IgM and IgG in the spectral region from 550nm to 650 nm:

Curve 1: (14058A3) Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgA.
Curve 2: (14058G4) Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgG.
Curve 3: (14058M2).Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgM.

(3). Summary of results

In general, the results were most encouraging, even with low signal strength, and demonstrate the robustness of the technique. These tests provide strong indications that it is possible to differentiate between acute and chronic renal transplant rejection and cyclosporine toxicity.

ii. Applied to Heart Transplant Biopsies

Although traditional medical treatment modalities and newer surgical interventions may provide short-term relief, cardiac transplantation may still be the only intervention that can alter the natural history and poor prognosis for patients with end stage cardiomyopathy. In spite

of medical advances. rejection and infection of transplanted hearts still result in significant morbidity and mortality, and the development of graft coronary occlusive disease limits even longer-term survival. A particularly lethal from of rejection, called humoral rejection, usually occurs early after transplantation. The diagnosis that leads to correct therapy is best determined from an immunofluorescence evaluation of frozen sections of heart biopsies from the transplanted heart.

Non-rejection pathology is frequently seen post transplant to include ischemia or catecholamine effects, interstitial fibrosis, myocardial calcification, and cyclosporine-associated endocardial infiltrates called the Quilty effect. It is therefore very important to be able to automatically diagnose evidence of immunosuppressive disorders even if there is no evidence of cellular rejection.

The International Society of Heart Transplantation (ISHT) provides criteria to enable grading of heart rejection ranging from Grade 0 to +4. Figure 12 shows two spectra from two patients, both negative for humoral rejection with an ISHT grate 0 and also negative for Quilty effect. Both were of right ventricular septal endocardial biopsies of heart transplant patients. The slides were stained with FITC conjugated antisera to immunoglobulins, the third component of complement and fibrinogen. Both spectra are fundamentally the same. The results summarized are:

Curve 1: (16757) Negative Quilty effect, negative rejection, ISHT Grade 0.

Curve 2: (16471) Negative Quilty effect, negative rejection, ISHT Grade 0.

This example provides a good indication that spectra are consistent between patients with similar findings.

In general, immunofluorescence studies with antibodies to immunoglobulin G, immunoglobulin A, immunoglobulin M, Clq, C'3, HLA-DR, and fibrinogen and immunoperoxidase staining for endothelial cells (factor VIII-related antigen) and macrophages (KP1 [CD68]) are regularly performed, and are enhanced with the present invention, because spatially resolved immunofluorescence features can be quickly, accurately and inexpensively studied and evaluated. The spectrograph, in conjunction with neural network powered data analysis, provides an immediate and digitally objective interpretation of spectral objects present in such tissue to aid the pathologist in making an assessment. Thus, the spectroscope/microscope combination enhances the speed and specificity of early indications of organ rejection.

2. Applied to Other Fluorescent Techniques

As stated above, biological matter be stained with a fluorescent dye or labeled with a fluorescent tag. Immunohistochemical reactions comprise one category of reactions and include mononclonal and polyclonal antibody staining with immunohistologic markers. Fluorescent in situ hybridization (FISH) is another category and can be directed to genetic analysis or protein analysis. In the former, hybridization takes place between a nucleotide label probe (called the "antisense strand") and an endogenous nucleotide (e.g. mRNA, called the "sense strand"). This is referred to as a paired-nucleotide interaction. In the latter, proteins are labeled and incubated with tissue that includes the target binding protein in what is called a protein-protein interaction. Examples of the application of the present invention for the diagnosis of disorders resident in such matter is now set forth.

a. Applied To Cervicovaginal Disorders

Effective diagnosis of two particular disorders, namely Human Papilloma Virus (HPV) and *Chlamydia Trachomatis* (CT), has become a subject of increasing importance to pathologists. The first disorder is believed to be a precursor of cervical cancer, and the second disorder is the most common sexually transmitted bacterial pathogen in the U.S., and is recognized to cause substantial morbidity. However, the diagnosis of both disorders are subject to the aforementioned limitations in technology. PAP smears currently screen for the early detection of cervical cancer and other abnormalities by preparing slides of stained, exfoliated cervical cells for analysis on a light microscope. While a valuable screening tool, PAP smears detect only 50-80 % of the abnormalities subsequently found by histopathological examination of biopsy specimen. Further, these two disorders are not successfully tested for with the conventional PAP smear screening.

Siadat-Pajouh et al. and others have shown that cancer-associated HPV genotypes in the nucleus of cervicovaginal cells are detectable by DNA analysis of the cells in a PAP smear slide via use of a fluorescence based in situ hybridization (FISH) assay. Images of the cells acquired (filtered) with a fluorescent microscope are then digitally captured by a CCD camera, and then analyzed using algorithms which detect all cell nuclei from images of the DNA counterstain. The images of the nuclei can then be used as a mask and mapped over the FISH image of the same microscopic scene to quantify the corresponding fluorescent HPV signal from each nucleus. (See *Siadat-Pajouh et al.*, "Detection of HPV Type 16/18 DNA in Cervicovaginal Cells by Fluorescence Based In Situ Hybridization and Automated Image Cytometry," *Cytometry*, 15:245-257 (1994)). This method can be described as a single wavelength examination of fluorescent signals with a single wavelength filter. To gain more

data about the DNA structures of the nucleus resulting in enhanced speed, specificity and accuracy of HPV identification, multiple fluorescent tags must be used which would require the use of multiple filters for the fluorescent microscope. However, as discussed above, single wavelength examination with multiple filters is time consuming, risks sample bleaching, and throws away useful multi-wavelength data.

Further, while detection of Chlamydia Trachomatis (CT) , the most common sexually transmitted disease in the U.S., has been difficult using standard PAP smear techniques, direct immunofluorescence (DIF) stainings of smears with fluorescein-conjugated monoclonal antibody has been shown to be effective in its diagnosis. (See *Garozzo et al.*, "Chlamydia Trachomatis diagnosis: a correlative study of pap smear and direct immunofluorescence,", *Clin. Exp. Obst. Gyn.*, vol. 20(4):259-266 (1993).)

The present invention has utility as a low cost, rapid and efficient diagnostic tool and method that could assist in the identification of these "early risk" disorders together with (in addition to) the PAP smear screening, all in a single test. In particular, the present invention can automate the analysis of normal and diseased cells that are identifiable using a sensitive FISH assay and DIF staining, to more objectively, cost effectively, and rapidly identify the presence of HPV and CT from a PAP smear.

i. Identification of Human Papilloma Virus (HPV)

One primary goal of new cytology instruments is to reduce the false-negative ratio (FNR) of screened PAP smears. Of the various techniques offered to increase the detection rate of cytologically undetected false-negative cases, screening simultaneously for HPV in the DNA of epithelial cells along with PAP smears has been shown to be one of the most effective. When used together, they appear to complement each other and offer a high detection rate.

The reason is that substantial evidence has accumulated associating specific HPV's with human anogenital disorders, most notably cervical cancer. To date, more than 60 HPV genotypes have been described, and about 20 of these are associated with anogenital lesions. These 20 can be characterized into "high risk" and "low risk" groups, in accordance with their association with benign and malignant tumors. As detailed in the *Siadat-Pajouh et al.* article, referred to above, a FISH assay has been developed to detect HPV 16 and 18 in cervical smears, these two sequences being most identified with malignant lesions. The speed, specificity and accuracy of multiple HPV type identification can be greatly enhanced by the use of multiple fluorescent tag probes. While the conventional FISH assay is one relatively low cost method of HPV identification, single wavelength examination of multiple fluorescent signals, with an

equal number of wavelength filters (filters), is time consuming, risks bleaching the sample, and discards the multi-wavelength data that define a fluorescent feature.

The multispectral topography system is a tool that enables the use of such multiple tagging by collecting entire spectra and using deconvolution algorithms to accurately differentiate the various spectral signals that contribute to an observed fluorescent envelope.

ii. Identification of Chlamydia Trachomatis (CT)

As discussed above, among the techniques used for identifying CT, DIF has been found to be a very effective technique. In particular, CT specific monoclonal antibodies, conjugated to fluorescein isothiocyanate provides highly specific fluorescein-staining. This test is well suited to sampling and handling similar, and, as with the FISH assay, is consistent with that of a PAP smear.

iii. The Dual Staining Method

Conventional FISH and DIF assays require a high quality fluorescent microscope equipped with special objectives and multiple filters. Testing for these conditions thus makes these techniques inappropriate for most office settings. However, using the multispectral imaging system of the present invention, a single cervical PAP smear slide that is dual-stained with FISH labeling and DIF staining can be automatically and simultaneously diagnosed for both HPV sequencing and CT identification by collecting all of the wavelength data that define these conditions.

b. Tissue Infected with Tuberculosis Bacilli

The analysis of matter stained with fluorescing dyes such as rhodamine/auramine (exogenous fluorphores) is greatly enhanced with the application of the present invention. The following is a description of one such experiment.

Slides of tissues from patients either known to be infected with tuberculosis (TB) and also free from TB were analyzed. The slides were prepared with Auramine/Rhodamine stain in preparation for observation under an Olympus BH2 epi-fluorescence microscope. The excitation wavelength was 510 nm and spectra were acquired through a long pass filter that allowed all light of greater than 540 nm to pass, and blocked the excitation wavelength and all wavelengths shorter than 540 nm.

FIG. 4 shows the video image of a calibration smear known to be positive for TB coded cTB11299sp3. The "observed" image camera uses false color enhancement for easy identification of areas in the smear with high intensity emission. FIG. 5 shows the spectral file acquired by the spectrograph, also with false color enhancement, as observed on the computer monitor. Each acquisition captures a slice of the smear, as shown by the rectangular box in

FIG. 4. Objects marked 1 and 2 correlate with the spectral image in FIGS. 5 and 6 and the spectra in FIG. 7. The video camera of the system had no difficulty in observing areas of intense light for those samples with TB and a noticeable absence of light in samples without TB. FIG. 6 shows the spectral image partitioned into "channels". Each channel is the spectrum of an area in the slice. For simplicity in presenting this concept, only 25 channels are shown representing an area on the smear of 8 by 2.5 μm.

Under normal operating conditions there are a maximum of 240 channels, each channel corresponding to one row of pixels. At this resolution, each of 240 objects is 1 μm by 2.5 μm when submitted with a 20X objective, and are all acquired simultaneously. Partitioning is set-up either by the operator or automatically by the computer. If a filter set is in the system, blocking wavelengths below a certain value, it is usual to establish channels to eliminate the bulk of absent wavelength data. Each spectrum corresponds to a specific location on the sample smear that correlate to channel number. The Y-axis provides signal intensity that can be calibrated to quantify the signal present against the object emitting the signal. The bands in FIG. 6 indicate the location of identical spectra that can be painted back to the original observed image. Each of these spectra indicate the presence TB and were identified with the USNN described in the following section.

In most fluorescence experiments, fluorescent signals are composed of natural fluorescence as well as from "tags". It was found that signal intensity levels of a smear positive for TB would greatly exceed the signal from natural fluorescence (also referred to as autofluorescence). The system produces a very large number of spectra simultaneously, many of which can be different from its neighbor, and indicative of differences in condition or state at a particular location.

To illustrate how the USNN concept works for the identification of TB, the calibration smear ctb11299sp3 was used to train the USNN to recognize the spectral characteristics of a smear presenting TB, as previously shown in FIGS. 4, 5, 6, and 7. Spectral and observed images were then captured from a smear known to be positive for TB, coded as tb12110sp2. The same channel selection was used as for the calibration acquisition.

FIG. 8 shows the USNN presentation of the analysis of the calibration smear, ctb11299sp3, and the patient sample coded tb12110sp2. The top left indicates that the first acquisition was a training set and there were 9 spectral features identified. If the threshold (sensitivity) of the USNN were adjusted, then it would locate either fewer spectra, by only looking for gross differences, or more spectra by looking for subtle differences.

The first column shows channel number, the second the spectral object associated with that particular channel. The third and fourth columns show the search for recognized spectral objects in tb12110sp2. A pathologist confirmed that the area-emitting object 1 was clearly that of TB. The fourth column indicates that there are 12 recurrences of object 1, 3 occurrences of object 8 and some spectra that tend toward object 8. The numbers such as NM0.0607823 indicate the percent similarity to the object indicated. Considering the signal strength of object 8, it is probably a small amount of natural fluorescence that is expected to be present. The spectral intensity and image data provides enough information for quantification and digitization.

Having thus described the basic principles and exemplary embodiments of the invention, it will be apparent that further variations, alterations, modifications, and improvements will also occur to those skilled in the art. For example, it is understood that the system may be designed with modified optics to capture a wider or different range of wavelength spectra than that identified above. Further, it is understood that the present invention is not limited to the use of microscopes. The spectrography subsystem may operate with any type of light image transmitter capable of transmitting an image mammalian cells and tissue, such as any lens based, telescopic, system, or a fiber optic based imaging system, such as an endoscope. Further, the matter is not limited to prepared slides. For example, the system could automatically analyze the spectral characteristics of cervico-vaginal tissue during an actual gynecological examination by connecting the spectroscopy subsystem and computer to a colposcope, for example. Finally, this system is not limited to use as a primary diagnostic tool. It may also be used as a tool to improve quality control in the pathology lab. For example, during screening of PAP smears, in the event that a cyto-technician observes a specimen from a particular patient with abnormal or atypical cell characteristics, while previous specimens were reported as "negative," all previously screened samples should be reviewed by a cytopathologist to ascertain that no "false negative" reporting has occurred. Further, in some labs, a certain percentage of "negative" samples may be submitted for rescreening by a senior cytopathologist. The system of the present invention may be used to rescreen such samples, thus improving quality assurance by increasing objectivity of analysis (by reducing dependence on human judgment) as well as increasing the speed and lowering the costs of the screening/rescreening process. Accordingly, the foregoing discussion is intended to be illustrative only; the invention is limited and defined only by the various following claims and equivalents thereto.

CLAIMS

1. A multispectral topography system for automatically assessing mammalian matter for evidence of disease, the system comprising:

an image transmitter having an optical output and a source of light that illuminates the matter, the transmitter adapted to transmit an image of a section of the matter to the optical output;

a multispectral imaging spectroscopy subsystem connected to the optical output, wherein the subsystem substantially simultaneously spectrally disperses the transmitted image into multiple component wavelengths to create a spectral image; and

a processor that processes the spectral image to provide diagnostic data representative of the image.

2. A multispectral topography system for automatically assessing mammalian matter for evidence of disease, the system comprising:

an image transmitter having an optical output and a source of light that illuminates the matter, the transmitter adapted to transmit an image of a section of the matter to the optical output;

a multispectral imaging spectroscopy subsystem connected to the optical output, the subsystem including

an imaging spectrograph having an entrance slit that permits the passage of light from a slice of the transmitted image of the section of the matter and a spectrum dispersing prism and mirror arrangement that disperses the light passed through the entrance slit into multiple component wavelengths of a predetermined spectral range to create a spectral image, and

a first charge-coupled-device (CCD) camera coupled to the spectrograph that acquires, and prepares the spectral image; and

a computer subsystem including a data processor that processes the prepared spectral image and diagnostic data representative of the slice of the image.

3. The system of claim 1 or 2, wherein the matter to be assessed are in vivo.

4. The system of claim 1 or 2, wherein the image transmitter is a lens-based image magnification system.

5. The system of claim 4, wherein the matter comprises a pathology specimen prepared on a slide and the image transmitter is a microscope that transmits the image of a magnified section of the specimen to the optical output.

6. The system of claim 5, wherein the microscope includes an x-y stage capable of sequentially moving the specimen so that the entrance slit of the spectrograph permits the passage of light from adjacent slices of the specimen.

7. The system of claim 6, wherein the x-y stage is automatically controlled by the computer.

8. The system of claim 2 or 4, further including an image director disposed between the image transmitter and the spectroscopy subsystem and having a first output in optical communication with the entrance slit of the spectrograph and a second output in optical communication with a second CCD camera that captures an observed image of the section of the specimen.

9. The system of claim 8, wherein the image director is a beam splitter that alternatively directs the image of the specimen to the spectrograph or the second CCD camera.

10. The system of claim 8, wherein the image director is a beam splitter cube that simultaneously directs the image of the specimen to the spectrograph and the second CCD camera.

11. The system of claim 5, wherein the specimen is a tissue biopsy.

12. The system of claim 5, wherein the specimen is a cell smear.

13. The system of claim 5, wherein the optical output of the microscope includes a standard camera interface and the imaging spectrograph connects to the interface.

14. The system of claim 2, wherein the data processor comprises an unsupervised neural network.

15. The system of claim 2, wherein the data processor comprises a supervised neural network.

16. The system of claim 2, wherein the data processor comprises an unsupervised neural network component and a supervised neural network component.

17. A multispectral topography system for automatically assessing mammalian matter for evidence of disease, the system comprising:

means for illuminating the matter;

means for transmitting an image of a section of the matter to an optical output;

means for simultaneously spectrally dispersing the image into multiple component wavelengths of a predetermined spectral range;

means for acquiring the dispersed image from the dispersing means and preparing the acquired image; and

means for processing the prepared image with a neural network to provide a diagnosis of the matter.

18. A method of spectrally analyzing mammalian matter for the presence of disease based upon a spectral analysis of the morphologic and physiologic deviation of the matter from the norm, the method including:

illuminating the matter with a light source;

transmitting an image of the matter to a multispectral imaging spectrograph;

spectrally dispersing the transmitted image through a prism and mirror arrangement into multiple component wavelengths of a predetermined spectral range;

acquiring and preparing the spectrally dispersed image of the multiple component wavelengths; and

processing the prepared, spectrally dispersed image to provide a diagnosis.

19. The method of claim 18, further including providing a visual display of the observed image obtained at the optical output.

20. The method of claim 19, further including providing a visual display of the manipulated spectrally dispersed image.

21. The method of claim 18, wherein the processing implements a neural network.

22. A method of spectrally analyzing a pathology specimen for the presence of disease based upon a spectral analysis of the biological and functional deviation of the specimen from the norm, the method comprising:

transmitting a magnified image of a section of the specimen to an optical output;

permitting the light of a slice of the image to transmit through an entrance slit, the slice of the image comprising multiple objects;

spectrally dispersing the transmitted image of the slice through a prism and mirror arrangement into multiple component wavelengths of a predetermined spectral range;

acquiring and preparing the spectrally dispersed image of the multiple component wavelengths for processing; and

processing the prepared, spectrally dispersed image with a neural network to classify the slice of the image into one of a preset number of categories indicative of the pathological condition of the slice of the image.

23. The method of claim 22, further including providing a first visual display containing the spectrally dispersed image and a second visual display of the observed image.

24. The method of claim 22 or 23, further including:

(a) translating the specimen along an axis to permit the light of a slice of the image adjacent to the slice of the image previously transmitted to transmit through the entrance slit;

(b) spectrally dispersing the transmitted light of the adjacent slice of the image through a prism and mirror arrangement into multiple component wavelengths of a predetermined spectral range;

(c) acquiring and preparing the spectrally dispersed light of the adjacent slice of the image; and

(d) processing the prepared and spectrally dispersed light of the adjacent slice of the image with a neural network to classify each object in the adjacent slice of the image into one of a preset number of categories indicative of the condition of each object.

25. The method of claim 24, further including repeating (a), (b), (c), and (d) until the desired number of slices of the image of the specimen have been processed and providing a clinical diagnosis therefrom.

26. A multispectral topography system for automatically assessing the fluorescent characteristics of mammalian matter for evidence of disease, the system comprising:

an image transmitter having an optical output and a source of filtered light that illuminates, and is absorbed by, the matter, the transmitter adapted to transmit an image of fluorescent light reemitted from a section of the matter to the optical output;

a multispectral imaging spectroscopy subsystem connected to the optical output, wherein the subsystem substantially simultaneously spectrally disperses the transmitted image into multiple component wavelengths to create a spectral image; and

a processor that processes the spectral image to provide diagnostic data representative of the image.

27. A multispectral topography system for automatically assessing the fluorescent characteristics of mammalian matter prepared as a pathology specimen for evidence of disease, the system comprising:

a fluorescent microscope having an optical output and a source of filtered light that illuminates, and is absorbed by, the specimen, the transmitter adapted to transmit an image of fluorescent light reemitted from a section of the specimen to the optical output;

a multispectral imaging spectroscopy subsystem connected to the optical output, the subsystem including

an imaging spectrograph having an entrance slit that permits the passage of light from a slice of the transmitted image of the section of the specimen and a spectrum dispersing prism and mirror arrangement that disperses the light passed through the entrance slit into multiple component wavelengths of a predetermined spectral range to create a spectral image, and

a first charge-coupled-device (CCD) camera coupled to the spectrograph that acquires the spectral image, converts the image into digital data, and manipulates the data; and

a computer subsystem including a neural network processor that processes the manipulated data representative of the spectral image and provides diagnostic data representative of the pathologic condition of the slice of the image.

28. The system of claim 27, wherein the specimen is an endogenous fluorophore.

29. The system of claim 28, wherein the specimen includes an autofluorescent compound, such as elastin or collagen.

30. The system of claim 28, wherein the specimen is prepared with an immunofluorescent staining technique, including fluoresceinated antisera to immunglobulins.

31. The system of claim 27, wherein the specimen is treated with at least one fluorochrome.

32. The system of claim 31, wherein the specimen is treated with a histochemical probe labeled with a flourochrome.

33. The system of claim 32, wherein a fluorescent in situ hybridization is performed on the specimen and the processor provides data indicative of the presence of at least one strain of one of a genetic disorder, malignancy, bacteria and virus.

34. The system of claim 32, wherein the specimen is prepared with an immunohistochemical stain having immunohistological markers, including one of monoclonal and polyclonal antibodies conjugated to a fluorescent tag.

35. The system of claim 34, wherein the specimen is stained with a DIF assay and the processor provides data indicative of the presence of chlamydia trachomatis (CT).

36. The system of claim 32, wherein the specimen is stained with both a FISH assay and DIF assay and the processor provides data indicative of the presence of both HPV and CT.

37. A method of analyzing the fluorescent characteristics of mammalian matter for the presence of disease based upon a spectral analysis of the morphologic and physiologic deviation of the matter from a norm, the method comprising:

illuminating the matter with filtered light of a specified wavelength to cause the matter to fluoresce;

transmitting an image of a section of the fluorescing matter to an optical output;

spectrally dispersing the transmitted image of the slice into multiple component wavelengths of a predetermined spectral range;

acquiring and preparing the spectrally dispersed image of the multiple component wavelengths; and

processing the prepared, spectrally dispersed image with a processor to classify the slice of the image into one of a preset number of categories indicative of the condition of the slice of the image.

38. The method of claim 37, wherein the preparing of the spectrally dispersed image comprises digitizing the image and manipulating the digitized image with digital signal processing.

39. The method of claim 37, further including:

(a) translating the matter along an axis to permit the light of a slice of the image adjacent to the slice of the image previously transmitted to transmit through the entrance slit;

(b) spectrally dispersing the transmitted light of the adjacent slice of the image through a prism and mirror arrangement into multiple component wavelengths of a predetermined spectral range;

(c) acquiring and preparing the spectrally dispersed light of the adjacent slice of the image; and

(d) processing the prepared and spectrally dispersed light of the adjacent slice of the image with a neural network to classify each object in the adjacent slice of the image into one of a preset number of categories indicative of the condition of each object.

40. The method of claim 39, further including repeating (a), (b), (c), and (d) until the desired number of slices of the image of the matter have been processed and providing a clinical diagnosis therefrom.

FIG. 1A

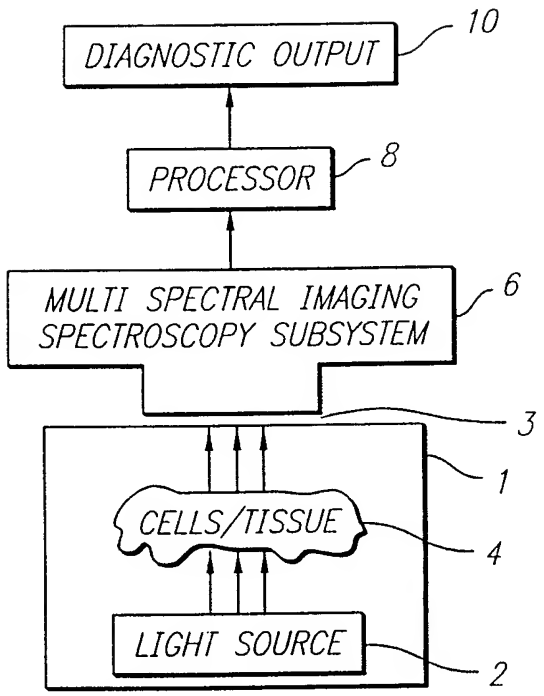

DIAGNOSTIC OUTPUT
10
PROCESSOR
8
MULTI SPECTRAL IMAGING SPECTROSCOPY SUBSYSTEM
6
3
1
CELLS/TISSUE
4
LIGHT SOURCE
2

FIG. 1B

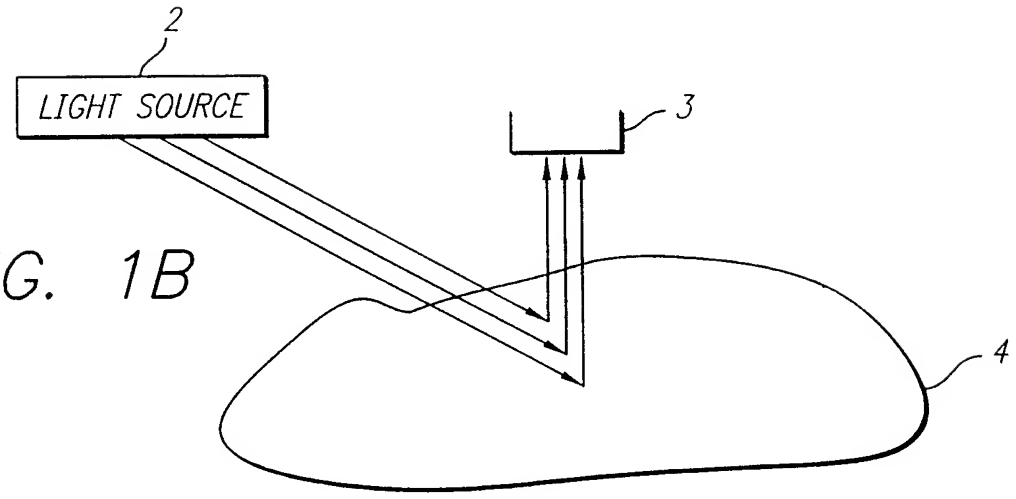

2
LIGHT SOURCE
3
4

FIG. 2

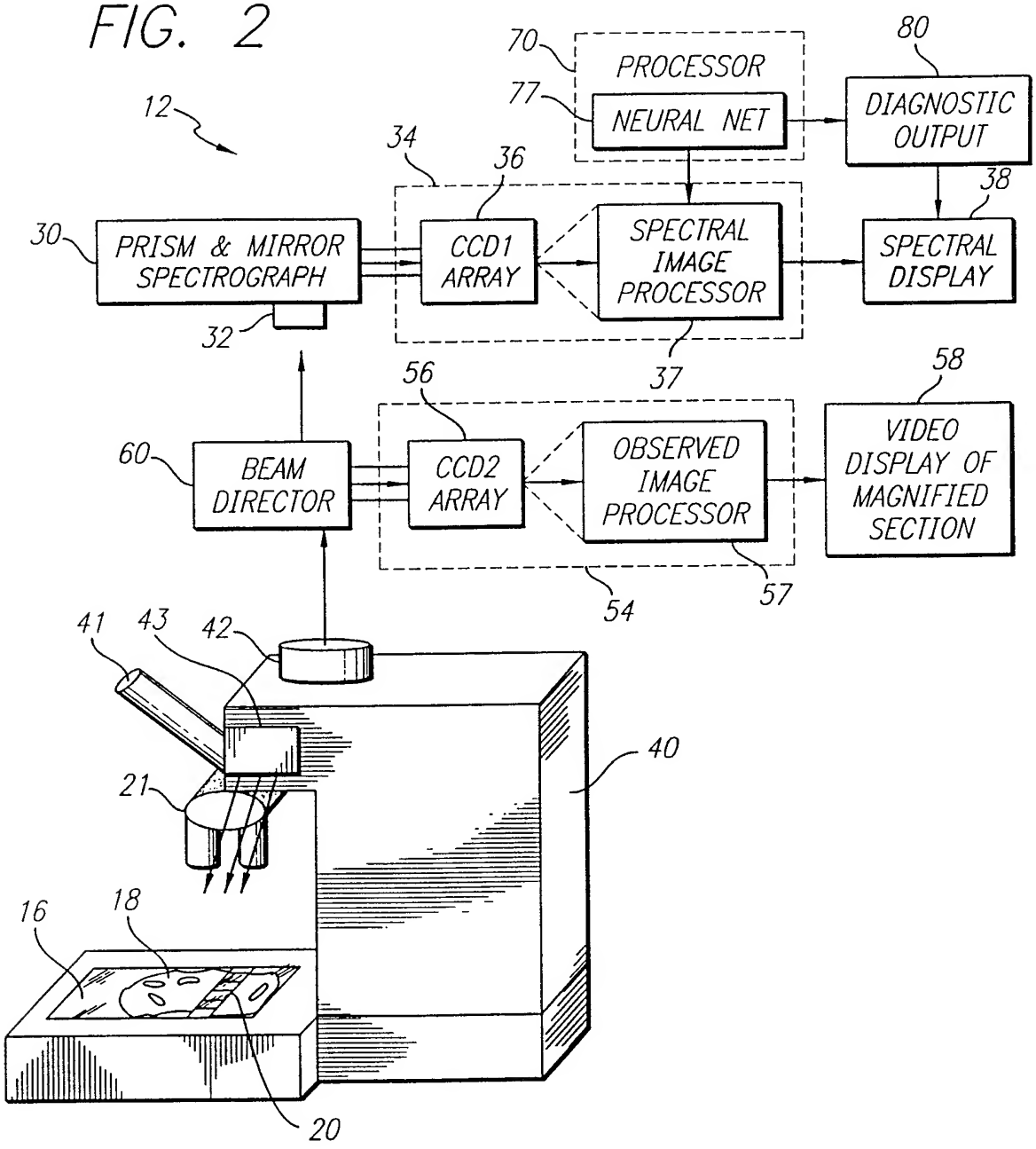

70
PROCESSOR
77
NEURAL NET
80
DIAGNOSTIC OUTPUT
12
34
36
38
30
PRISM & MIRROR SPECTROGRAPH
CCD1 ARRAY
SPECTRAL IMAGE PROCESSOR
SPECTRAL DISPLAY
32
37
56
58
60
BEAM DIRECTOR
CCD2 ARRAY
OBSERVED IMAGE PROCESSOR
VIDEO DISPLAY OF MAGNIFIED SECTION
54
57
41
43
42
40
21
16
18
20

FIG. 2A

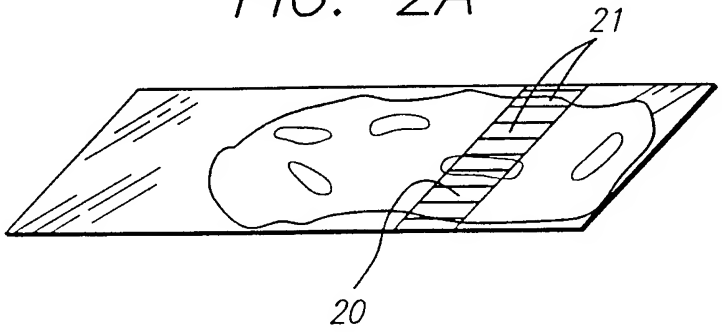
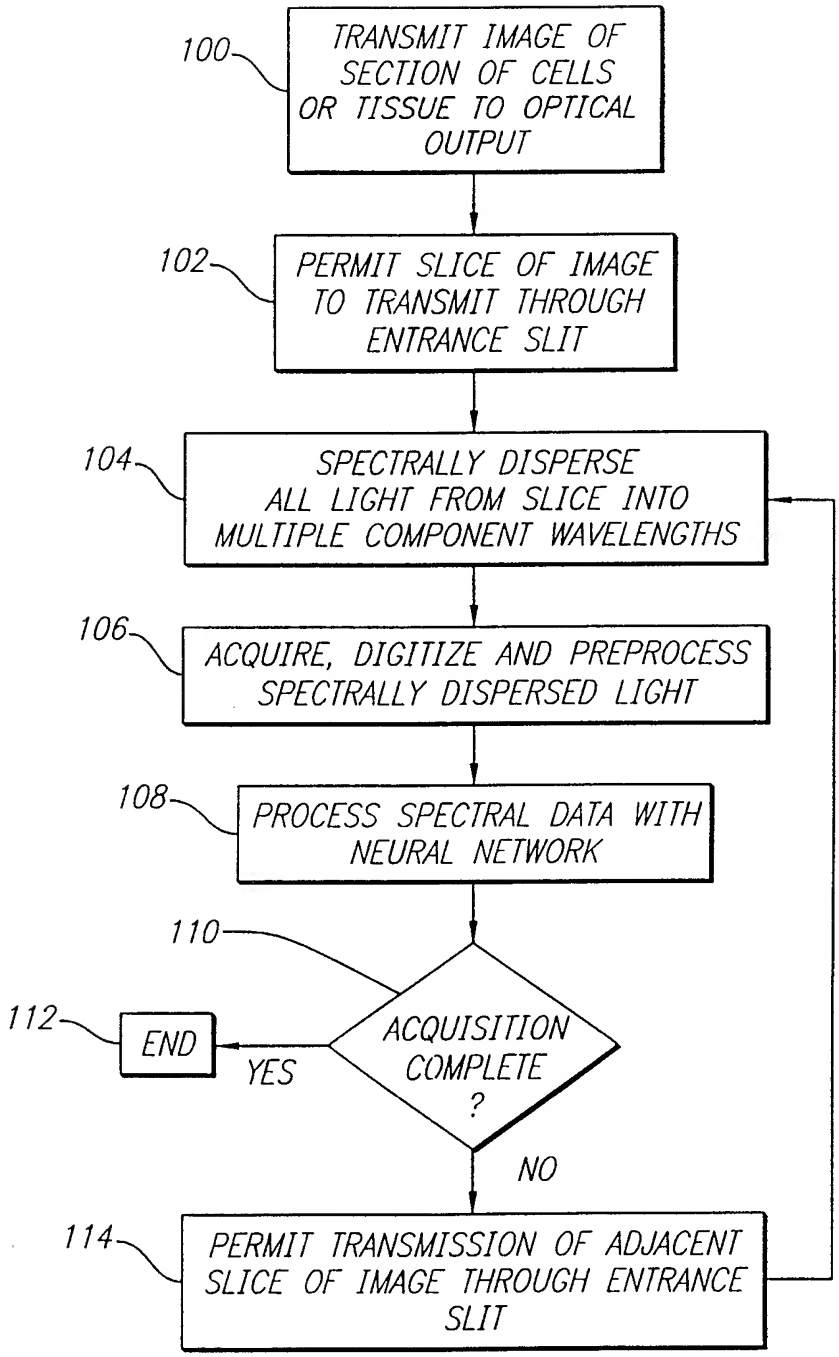

21
20

FIG. 3


100
TRANSMIT IMAGE OF SECTION OF CELLS OR TISSUE TO OPTICAL OUTPUT
102
PERMIT SLICE OF IMAGE TO TRANSMIT THROUGH ENTRANCE SLIT
104
SPECTRALLY DISPERSE ALL LIGHT FROM SLICE INTO MULTIPLE COMPONENT WAVELENGTHS
106
ACQUIRE, DIGITIZE AND PREPROCESS SPECTRALLY DISPERSED LIGHT
108
PROCESS SPECTRAL DATA WITH NEURAL NETWORK
110
ACQUISITION COMPLETE ?
112
END
YES
NO
114
PERMIT TRANSMISSION OF ADJACENT SLICE OF IMAGE THROUGH ENTRANCE SLIT

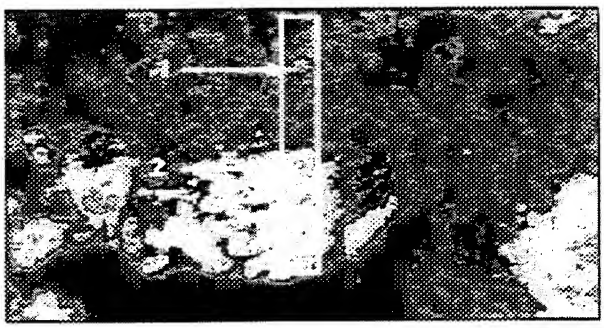


FIG. 4

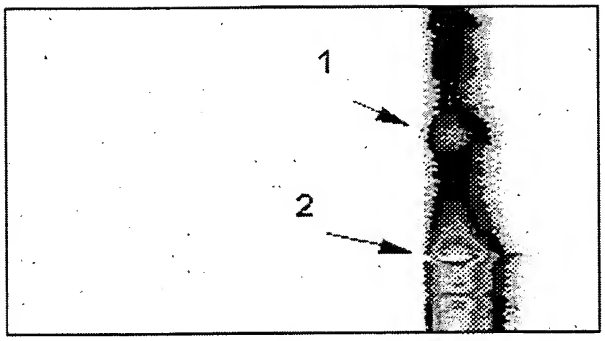


FIG. 5

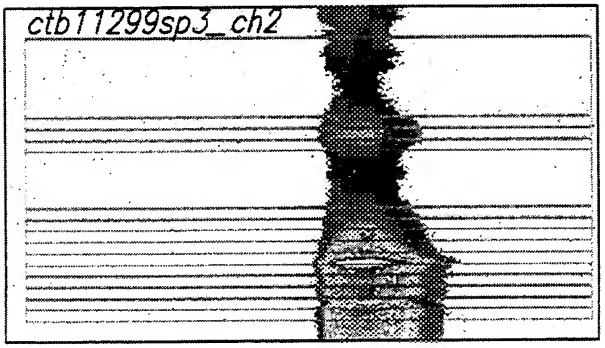


FIG. 6

FIG. 7

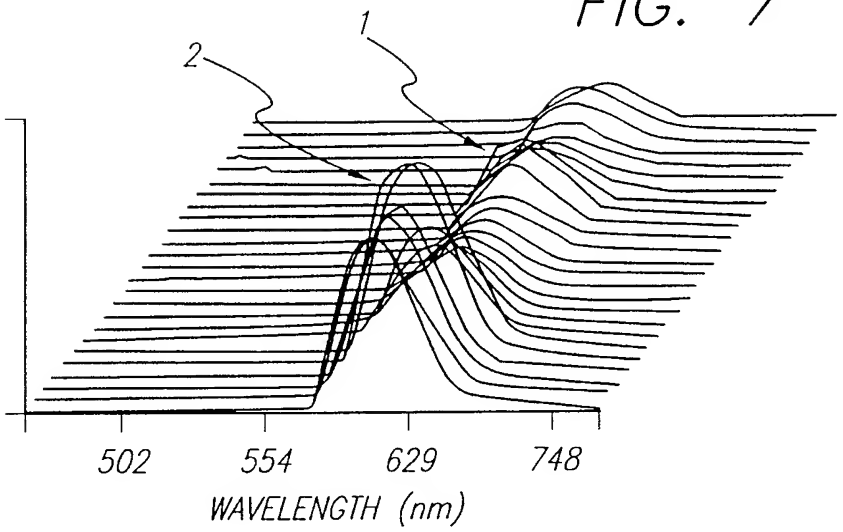


FIG. 8

USNLearn 3 ctb11299sp3_ch1.3D		USNRecognize 4: TB12110sp2_ch2.3D	
No. of Spectral Features:9		No. of Spectral Features:11	
Subchannel No	Spectral Features	Subchannel No	Spectral Features
1	Object_0	1	Object_1
2	Object_6	2	Object_8
3	Object_6	3	Object_1
4	Object_6	4	Object_8
5	Object_6	5	Object_8NMO.485354
6	Object_4	6	Object_8NMO.0105921
7	Object_2	7	Object_8NMO.0102858
8	Object_8	8	Object_8NMO.82303
9	Object_8	9	Object_1
10	Object_8	10	Object_1
11	Object_8	11	Object_1
12	Object_3	12	Object_1
13	Object_5	13	Object_1
14	Object_6	14	Object_8
15	Object_1	15	Object_1_1NMO.897868
16	Object_1	16	Object_8NMO.0607823
17	Object_1	17	Object_1NMO.911283
18	Object_1	18	Object_1
19	Object_1	19	Object_1
20	Object_1	20	Object_1_0NMO.869678
21	Object_1	21	Object_1_1NMO.89892
22	Object_6	22	Object_1
23	Object_6	23	Object_1
24	Object_6	24	Object_1

FIG. 9

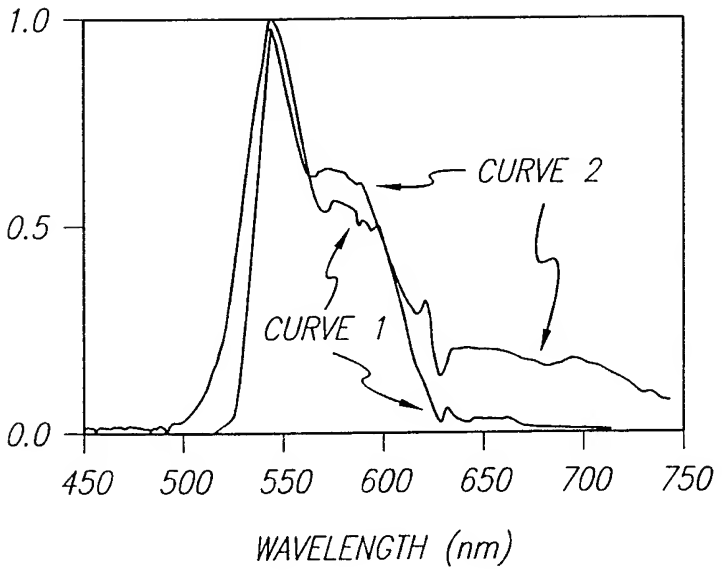

1.0
0.5
0.0
CURVE 2
CURVE 1
450
500
550
600
650
700
750
WAVELENGTH (nm)

FIG. 10

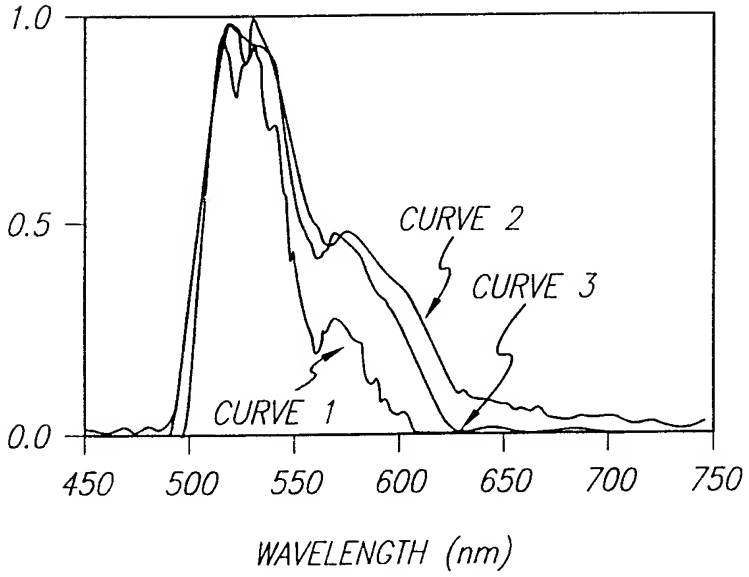
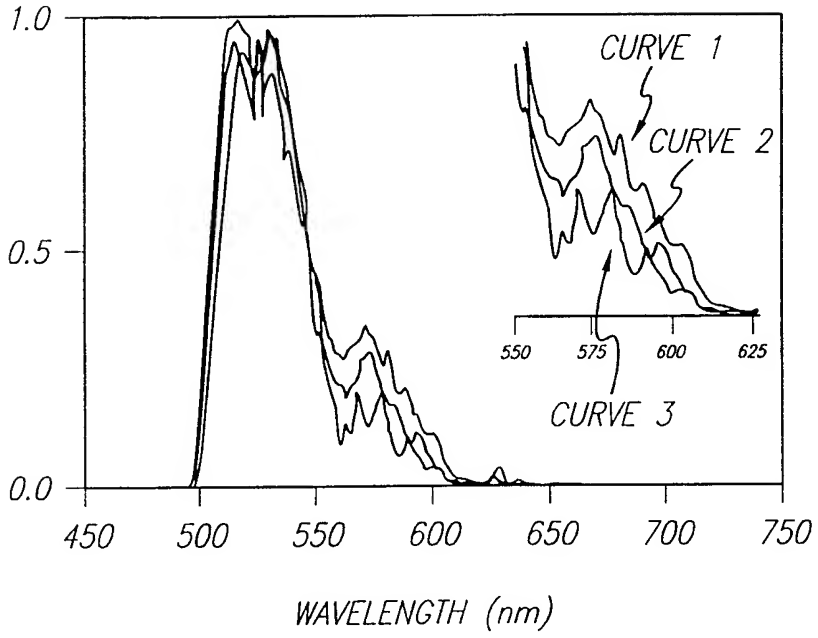

1.0
0.5
0.0
CURVE 2
CURVE 3
CURVE 1
450
500
550
600
650
700
750
WAVELENGTH (nm)

FIG. 11


1.0
0.5
0.0
450
500
550
600
650
700
750
WAVELENGTH (nm)
CURVE 1
CURVE 2
CURVE 3
550
575
600
625

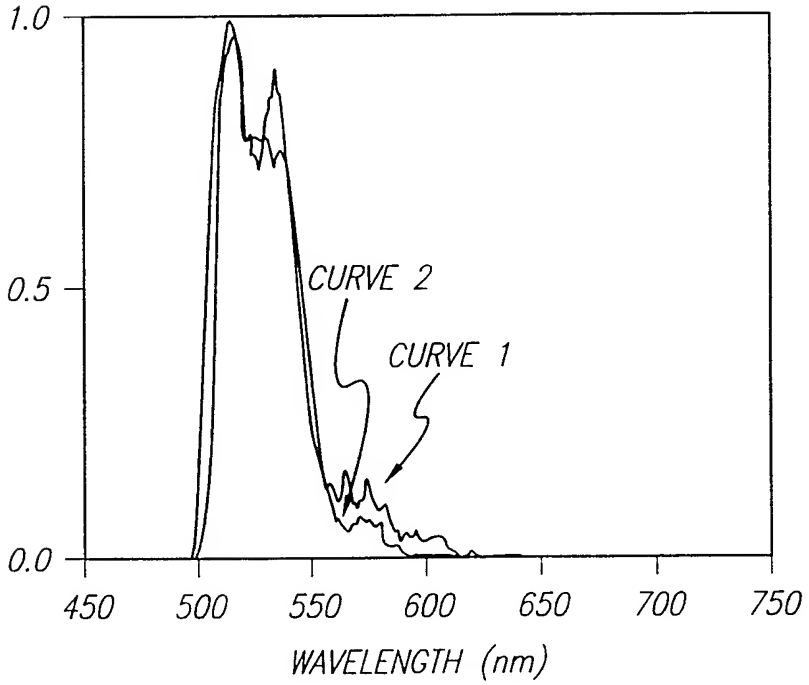

1.0
0.5
0.0
450
500
550
600
650
700
750
WAVELENGTH (nm)
CURVE 2
CURVE 1

FIG. 12

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 99/17004

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01J3/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 784 162 A (BUCKWALD ROBERT A ET AL) 21 July 1998 (1998-07-21) column 1, line 1 -column 3, line 28 column 5, line 5 -column 6, line 53 column 56, line 45 - line 53 column 58, line 54 -column 59, line 38	1-40
X Y A	US 5 528 368 A (LEWIS EDGAR N ET AL) 18 June 1996 (1996-06-18) column 7, line 33 -column 10, line 39; claim 1; figures 2,6	1,4-13, 17-21,26 2,14-16, 18-21,27 22,23,27
Y A	US 5 424 827 A (HORWITZ BRUCE A ET AL) 13 June 1995 (1995-06-13) column 2, line 13 - line 51 column 5, line 38 -column 6, line 14; figure 6	2,14-16, 18-21,27 22,39

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 1999	18/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Scheu, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/17004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 02483 A (UNIV YALE) 23 January 1997 (1997-01-23) page 1, line 1 - line 25; figure 5 -----	1,2, 18-20,26 17,22, 27-39

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inte onal Application No
PCT/US 99/17004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0830564 A	25-03-1998
		JP 11500832 T	19-01-1999
		WO 9721979 A	19-06-1997
		US 5936731 A	10-08-1999
		US 5817462 A	06-10-1998
		US 5719024 A	17-02-1998
		US 5856871 A	05-01-1999
		US 5835214 A	10-11-1998
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US 5424827 A	13-06-1995	NONE	
WO 9702483 A	23-01-1997	US 5793049 A	11-08-1998
		AU 6343996 A	05-02-1997